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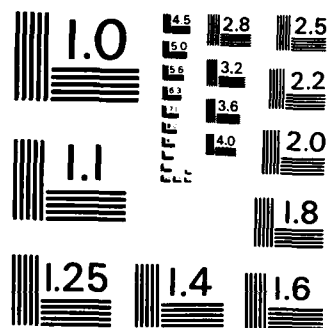
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# ONRL Report R-6-85

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Immunology Research in Israel

Claire E. Zomzely-Neurath

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## IMMUNOLOGY RESEARCH IN ISRAEL

Research in immunology has developed and flourished greatly in Israel. Initially, research in this area was carried out primarily at the Weizmann Institute of Science, Rehovot. In the late 1960s and 1970s, new academic centers were established for immunological research at universities in Tel-Aviv, Haifa, and Beer-Sheva. Today, Israeli industries use information from basic research in immunology to produce kits for clinical assays and various reagents for medical and analytical resources.

Important areas in immunology research being pursued by Israeli scientists include: investigation of immunoglobulin genes, structure-function analysis of antibodies and regulation of antibody production and expansion; genetics of autoimmunity and cancer; lymphokines and complement; autoimmunity; tumor immunology; transplantation and tissue typing; clinical immunology; infectious diseases; and applied immunology. This report describes the various facets of immunology research that have been studied in Israel and the scientists who are responsible for it.

### Background

In the 1920s and 1930s, several outstanding scientists were already active in areas related to immunology at the Hebrew University in Jerusalem. The parasitologists S. Adler and G. Mer, and the bacteriologist N. Kliegler made contributions that played a major role in the complete eradication of malaria from the endemic region of what was then called Palestine. In 1934, Dr. Chaim Weizmann founded the Daniel Sieff Research Institute. By 1947, this had expanded into what is today the multidisciplinary Weizmann Institute of Science. It was here that E. Katzir-Katchalski prepared synthetic polyamino acids and studied their properties as models for proteins. Sela and Katzir-Katchalski showed that attaching certain synthetic peptides to proteins may either decrease or significantly in-

crease their immunogenicity. By 1962, this had led to the development of synthetic antigens and their use for the elucidation of many facets of immunological phenomena, including the molecular basis of antigenicity and the establishment (in collaboration with H. McDevitt) of the determinant-specific genetic control of immune response and immunological responsiveness.

The Weizmann groups also made model oligopeptides, which were used for probing the antigen-binding sites of antibodies. R. Arnon, S. Fuchs, I. Schechter, and E. Mozes, students of Sela, were all involved in these early studies. In the early 1970s, these oligopeptides led to the concept of synthetic vaccines that had been first formulated by M. Sela and R. Arnon. D. Givol pioneered in Israeli studies of the chemical structure of the antibody molecule. I. Pecht was involved in the thermodynamic and kinetic analysis of antigen-antibody interactions.

At the same time, immunology on a more cellular level, with special relevance for cancer research, was already being studied extensively by M. Feldman and his collaborators. N. Haran-Ghera made a major contribution in the area of leukemogenesis, and N. Trainin discovered the thymic humoral factor. At the Hebrew University's Hadassah Medical School, A.L. Olitzki, O. Sultizeanu, and their colleagues made important contributions to the cellular aspects of immunology. M. Schlesinger became a pioneer in the field of histocompatibility antigens and was the first to show the theta antigen as a murine T-cell marker. In the late 1960s, D. Weiss (University of California, Berkeley) joined the Weizmann Institute to set up programs in immunopotential and cancer research.

A new center for immunology research was established at Tel-Aviv University in the late 1960s. Some of the researchers making important contributions at the Faculty of Science and the Medical School were I. Witz in tumor immunology, S. Ben-Efraim in delayed hypersensitivity, E. Pick in lymphokine

research, and J. Haimovich in immunoglobulin structure and biosynthesis.

#### Immunochemistry and Immunogenetics

Understanding the primary structure of the immunoglobulin (Ig) polypeptide chains was one of the main thrusts of research in immunochemistry in Israel during the 1960s and 1970s. D. Givol contributed to the identification of the variable domains of Igs, and I. Schechter provided a detailed understanding of the structure of antibody-binding sites and to the presence of the N-terminal (leader sequences) in the intracellularly located Ig chains. These studies paved the way, in the 1980s, to the investigation of the Ig genes, as well as detailed structure-function analysis of the antibodies and other immunologically important molecules and the regulation of antibody production and expression.

Structure-Function Analysis of Immunoglobulin Molecules. R. Laskow (Hebrew University, Hadassah Medical School, Jerusalem) and J. Haimovich (Department of Human Microbiology, Tel-Aviv University) have shown that the transition from the expression of membrane Ig to that of secretory Ig is due to a change in the transcription mode of the messenger RNA (mRNA) of these two types of Igs. Secretory IgM is produced in B-cells but is expressed to only a limited extent due to its degradation.

I. Pecht (Department of Chemical Immunology, Weizmann Institute) and his associates carried out the first mechanistic analysis of homogeneous antibody-hapten reactions. They were able to do a kinetic mapping of the antigen-binding site of the examined Ig and to resolve the conformational transitions induced in the antibody upon hapten binding. Through extensive studies of various homogeneous antibodies of different specificities, as well as heterologous chain recombinants of such molecules, they established a common mechanism. It involves two different conformational states of the combining site in each of its free and hapten-bound forms. Hapten binding causes a transition in these

equilibria, leading to the better binding conformation.

The longitudinal interactions among the Ig domains were first resolved through studies monitoring the circularly polarized fluorescence of intrinsic tryptophan residues, a method developed by I.Z. Steinberg and coworkers (Department of Chemical Physics, Weizmann Institute). Pecht et al. then developed a model system of the Ig light chain dimer derived from the IgA secreted by the MOPC 315 cell line. They found clear evidence for such longitudinal interactions--i.e., hapten binding at the variable regions of the chains was marked by changes at the constant domains.

The expression and fine specificity of idiotypic determinants have been studied in several systems. Monoclonal antibodies specific for the synthetic polypeptide antigen (T, G)-A--L were produced by E. Mozes (Department of Chemical Immunology, Weizmann Institute) and her associates. Their specificity and idiotype nature were compared to conventional antibodies. The role of monoclonal anti-idiotypic antibodies in T-cell regulation is currently being investigated.

Monoclonal antibodies against the *Torpedo* acetylcholine receptor (AChR) have been produced and analyzed by S. Fuchs and coworkers (Department of Chemical Immunology, Weizmann Institute) in their studies on experimental autoimmune myasthenia gravis (EAMG). These antibodies block AChR binding and sodium transport, accelerate the receptor turnover, and modify single channel properties. They can be used to follow antigenic changes in the AChR during muscle development and synaptogenesis. Antibodies to idiotypes on anti-AChR antibodies are being used to study the idiotype repertoire of the immune response to AChR and to assess the potential of anti-idiotypes for immune regulation of EAMG.

A. Zlotnick and D. Eilat (Laboratory for Autoimmune Diseases, Department of Internal Medicine, Hebrew University, Hadassah Hospital) are studying the structure and antibody specificity of

monoclonal antinuclear antibodies from SLE-prone mice and the immunochemical properties of autoantibodies to human albumin in different clinical syndromes.

An important innovation in immunochemistry was the use of synthetic polypeptides such as (T,G)-A--L to elucidate the genetic regulation of specific immune responses. This use was pioneered by M. Sela and collaborators (Department of Immunology and President, Weizmann Institute). For the first time, E. Mozes et al. showed with (Phe,G)-Pro--L that antibody specificity was genetically controlled in mice. They have recently demonstrated with synthetic T-cell replacing factors that immune response (Ir) genes may be expressed in different cell populations.

One of the keys to the mechanism of Ir gene function is the genetic restriction of cell-to-cell interaction. In this area, S. Ben-Sasson (Department of Immunology, Hebrew University, Hadassah Medical School, Jerusalem) and collaborators are studying the function and H-2 restriction of immortalized T-cell clones specific to ovalbumin by radiation leukemia virus transformation. Mozes et al. are studying interleukin-2-dependent T-cell clones specific to (T,G)-A--L. H-2 restriction of the interaction of T-cell clones with macrophages has been demonstrated in both systems. In both laboratories, studies are in progress to elucidate the genetic control of T (or helper factor)-B interactions for antibody production.

P. Lonaï (Department of Chemical Immunology, Weizmann Institute) has studied the Ia-associated antigen complexes that are released by macrophages and that contain part of the antigen in conjunction with Ia gene products. At present, the immunogenicity, binding restrictions, and biochemical characteristics of these "processed" antigens are being studied. J. Puri, in the same department, has analyzed the "immunogen" which is bound to the T-cells responding to different species' insulin. He has found that the binding of radioactive

antigen to these cells depends on the origin of MHC class II determinants of the antigen-presenting cells.

The avidin-biotin complex is being used by I. Cohen and A. Friedman (Department of Cell Biology) and C. Gitler (Department of Membrane Research) at the Weizmann Institute as a tool to study the molecular mechanisms by which antigens under Ir gene control are processed and presented to T lymphocytes. Differences of the response in the Ir phenotype to avidin could not be attributed to determinant selection by the antigen-presenting cells (APC). Further studies support the conclusion that restriction is due to the association between processed antigen and an APC element containing I-region products.

Genetics of Autoimmunity and Cancer. In I. Cohen's laboratory, the study of the regulation of experimental autoimmune thyroiditis (EAT) by mouse H-2 gene products led to the discovery of an H-2 mutation that influenced the EAT phenotype. R. Arnon and D. Teitelbaum (Department of Chemical Immunology, Weizmann Institute) have demonstrated that susceptibility to experimental allergic encephalomyelitis (EAE) is genetically controlled, both in guinea pigs and in mice. In the former, the sensitivity is inherited as a dominant trait and is linked to the major histocompatibility complex (MHC) of strain 1, but in mice there is no linkage with the H-2 locus.

H-2 linked resistance genes against leukemogenesis by four radiation leukemia viruses (Rad LV) have been investigated by N. Haron-Ghera and P. Lonaï (Department of Chemical Immunology, Weizmann Institute). All are dominant and map in the I region, suggesting that the Ir gene-controlled immune responsiveness of the host may be responsible, in part, for the preleukemic arrest of leukemogenesis.

Immunogenetics is important in the development of metastases. R. Laskov, (the Hebrew University, Hadassah Medical School, Jerusalem), M. Feldman (Department of Cell Biology, Weizmann

Institute), and S. Segal (Faculty of Sciences, Ben Gurion University, Beer-Sheva) have studied a sarcoma that originated in (H-2<sup>b</sup>×H-2<sup>k</sup>) F1 mouse (the T10 sarcoma). They found that tumor cells from local growth expressed only the H-2<sup>b</sup> halotypes, whereas cells from lung metastases expressed both halotypes. Clones of the T10 sarcoma expressed either H-2<sup>b</sup> halotypes or the two halotypes. Only the latter clones could metastasize.

The involvement of the K and I regions of the H-2 complex in resistance to hemopoietic allografts is being studied by G. Drislik (Department of Chemical Immunology, Weizmann Institute). His results suggest that class I (K and D) and class II (I-A and I-E) MHC genes, rather than hypothetical Hh genes, control hemopoietic resistance.

Human Ir Genes. Because genes in the human leukocytic antigen (HLA) system have a marked effect on susceptibility to a number of diseases, it is important to study HLA-linked Ir genes in man. Using the synthetic polypeptide antigens that helped to demonstrate Ir genes in mice and an *in vitro* system for T-cell activation, E. Mozes (Department of Chemical Immunology, Weizmann Institute), Z. Bentwich (Department of Internal Medicine, Kaplan Hospital, Rehovot), C. Brautbar (Hebrew University, Hadassah Medical School, Jerusalem), and coworkers have demonstrated the existence of immune response genes in man. Family studies indicated that the ability to respond to these antigens is inherited as an autosomal dominant trait linked to HLA. At least one of the genes controlling immune responsiveness to (T,G)-A--L and (H,G)-A--L seems to map between HLA-A and HLA-B. Patients with systemic lupus erythematosus cannot regulate their response to (T,G)-A--L; patients with thyroid autoimmune diseases can. They respond to this antigen like healthy people. Thus, different mechanisms of dysregulation seem to operate in different autoimmune diseases.

Studies in immunogenetics that were carried out in animals in the past are now being applied to the investigation

of the genetic regulation of the immune responses in humans. With the aid of newly developed methods, including those of molecular biology, this field is likely to promote progress toward treatment of human diseases in addition to understanding of the phenomena.

#### Lymphokines and Complement

E. Pick (Department of Human Microbiology, Tel-Aviv University) in the early 1970s established the first focus of lymphokine research. He studied the mechanism of the action of the migration inhibitory factor (MIF) at the level of the target macrophage. His laboratory was among the first to use sophisticated cell biology techniques for understanding lymphokine action. This led to the demonstration of the involvement of cyclic nucleotides, calcium, and the cytoskeleton in the effects of MIF.

Although not commonly considered a lymphokine (i.e., nonantibody lymphocyte products with potent biological activity), thymic humoral factor (THF) was discovered and characterized by N. Trainin and his colleagues (Department of Cell Biology, Weizmann Institute) in the mid-1970s. His group has been active in basic and applied studies of thymic hormones. These studies have now reached the level of clinical trials in immunodeficiencies and malignancies that are being performed at the Beilinson Hospital in Petah-Tiqva (Tel-Aviv) and the Kaplan Hospital.

Antigen-specific T-cell helper factors of both murine and human origin represent the principal thrust of research by the group headed by E. Mozes at the Weizmann Institute. Mozes established antigen-specific T-cell hybridomas and IL-2 dependent T-cell lines to serve as a source for helper factors. Cloned helper T-cells specific for (T,G)-A--L were shown to secrete an antigen-specific factor that expresses an idotype cross-reactive with that detected on the antibody of the same specificity. Monoclonal antibodies to the T-cells also react with the isolated helper factor. Studies on human antigen-specific helper factors are being



carried out by Mozes in collaboration with Z. Bentwich at the Kaplan Hospital in Rehovot. Of special interest is the use of T-cell factor production in response to (T,G)-A--L as an indicator of the immune status of patients with systemic or organ-specific autoimmune diseases.

S. Ben-Sasson, at the Hebrew University, Hadassah Medical School, Jerusalem, has also established antigen-specific T-cell clones in the mouse that act as helper cells *in vitro* and *in vivo*. The helper clones secrete an antigen-specific helper factor capable of triggering B-cells. Monoclonal antibodies recognize both the helper clone and its lymphokine product.

Interferons and Interleukins. Scientists at the Weizmann Institute have made major contributions in  $\alpha$ -interferon research. D. Wallach is actively working on the role of  $\gamma$ -interferon in modulating the expression of HLA antigens and as an enhancer of cytotoxin production. Wallach's laboratory is also involved in research on human tumor necrosis factor that led to the development of monoclonal antibodies useful for the purification of the factor. Also at the Weizmann Institute, M. Rubinstein and D. Fisher are performing interesting studies on the molecular characterization of human  $\gamma$ -interferon and its role as a monocyte-activating factor.

The clinical studies on  $\gamma$ -interferon in S. Levin's laboratory at the Kaplan Hospital, Rehovot, cover aging, IgE-type allergic diseases in children, immunodeficiencies, and cancer.

Israel was a latecomer to the explosion in interleukin research. This is rather surprising because Israeli scientists made pioneering contributions to the establishment of hemopoietic and lymphoid cells in long-term culture. Although interleukin 2 (IL-2) is widely used as a growth factor in laboratories throughout Israel, the major Israeli contribution to interleukin research has been in the area of molecular biology. R. Kaempfer, at the Hebrew University, Hadassah Medical School, Jerusalem, was the first to report the induction of

mRNA-encoding IL-2 (and  $\gamma$ -interferon) in human lymphocytes and their expression in *Xenopus laevis* oocytes. Kaempfer's group has also described two repression mechanisms involved in the induced expression of the human IL-2 gene. One of the two repressors was activated by the interaction between helper and suppressor lymphocytes. At the more applied level, E. Kedar, also at the Hadassah Medical School, is using IL-2 to propagate T lymphocytes sensitized *in vitro* against tumor cells. These cells are used for a combined immunotherapy *in vivo* of poorly immunogenetic tumors.

Studies of interleukin 1 (IL-1) are being carried out by A. Treves and co-workers at the Department of Oncology, Hebrew University, Hadassah Medical School, Jerusalem. They have developed several human macrophage cell lines by somatic hybridization (human-mouse) or immortalization of myelomonoblastic leukemia cells. Some of these cell lines produce IL-1, an IL-1 inhibitor and colony stimulating factors.

The study of lymphokines in Israel, as elsewhere, is becoming more biochemical. This is illustrated by studies of lymphokine synthesis at the DNA and mRNA level, by the use of sophisticated lymphokine purification techniques, and by investigations on the effect of lymphokines on target cell metabolism. This last aspect has engendered close links between lymphokine research and immunopharmacology. E. Pick, for example, at the Laboratory of Immunopharmacology, Sackler School of Medicine, Tel-Aviv University, is studying the effect of macrophage activating factor on enzymes involved in oxygen radical production and scavenging, and on arachidonic acid metabolism of macrophages. E. Razin, at the Hadassah Medical School in Jerusalem, is investigating the biochemistry of E-type mast cells. Razin has also contributed to the characterization of interleukin 3.

Complement research is an important area of immunology that is well developed in the US, Germany, and the UK, but that had not been emphasized in Israel. However, Z. Fishelson at the Weizmann

Institute is now focusing his interest on a key enzyme of the alternative pathway of complement, the C3/C5 convertase, that is being studied in fluid phase and on cell surfaces by a number of approaches.

#### Tumor Immunology

Controlling Tumor Growth and Progression. Mechanisms of leukemogenesis and characterization of leukemic cells are being studied by N. Haron-Ghera (Department of Chemical Immunology, Weizmann Institute). He has observed the appearance of leukemic cells prior to overt leukemia and demonstrated that both leukemic and preleukemic cells express TL antigens. The host's immune response against preleukemic cells has a part in establishing the latent period prior to the appearance of tumors. Susceptibility to the induction of leukemia-radiation-leukemia viruses is controlled by Ir genes.

T. Umiel (Hematology Department, Beilinson Hospital, Petah-Tiqva) is examining the occurrence of T,B and myeloid-cell markers in acute childhood leukemia and lymphoma. Various subtypes of leukemia correspond to one or another stage of lymphoid or myeloid cell differentiation pathways. Null cell leukemia expresses only early B-cell differentiation antigens. A single clone of cells from infant leukemia has been found to express both myeloid and B-cell markers. These studies are helping in the diagnosis, prognosis, and understanding of normal T,B and myeloid differentiation pathways.

Immunological aspects of the metastatic process are being studied by the groups of M. Feldman (Department of Cell Biology, Weizmann Institute) and S. Segal (Faculty of Health Sciences, Ben Gurion University, Beer-Sheva). They have found that the metastatic phenotype of a neoplastic gene is controlled by the relative expression of MHC genes coding for the class I antigens and that induced modulation of gene expression, including gene transfection, changes the metastatic capability of neoplastic cells. Somatic cell fusion between non-

metastatic tumor cells and lymphocytes resulted in hybrids with metastatic properties.

The role of macrophage cytotoxicity in controlling tumors is being investigated by two groups. R. Gallily and colleagues (Department of Microbiology, Tel-Aviv University) have demonstrated that *Mycobacterium orale* induces *in vitro* macrophage-mediated cytotoxicity against various tumor cells. The degree of malignancy was not correlated with susceptibility to cytotoxicity by activated macrophages. Macrophage-mediated cytotoxicity was correlated in many experimental systems with the production of a cytotoxic factor that resembles tumor necrosis factor. Another group at Tel-Aviv University, led by Y. Keisari, found that the susceptibility of tumor cells to the cytotoxic effect of macrophages was increased by antineoplastic drugs.

I.P. Witz and colleagues (Department of Microbiology, Tel-Aviv University) have demonstrated that the association of immunoglobulins with tumors is correlated with the occurrence of Fc receptors on nonlymphoid tumors. Passive administration of naturally tumor-reactive monoclonal antibodies present during the precancerous period significantly altered the carcinogenicity of urethan.

The study of the regulation of the immune response by neoplastic cells is another research area being pursued in Israel. D. Naor's group, Hebrew University, Hadassah Medical School, Jerusalem, has found that tumor cells contain immunogenic and suppressogenic molecules, and efforts are being made to isolate and characterize these entities. G. Berke (Department of Cell Biology, Weizmann Institute) is studying the structure and lytic functions of cytotoxic T-cells, focusing on the analysis of the recognition phase, as distinct from the lytic phase, in the interaction between the lymphocytes and target cells.

Tumor Diagnosis. The identity of antigens in immune complexes in neoplasia and the nature of a lymphocyte-determined membrane antigen in Epstein-Barr

Virus (EBV) transformed cells is being investigated by D. Sulitzeanu and coworkers (Department of Immunology, Hebrew University, Hadassah Medical School, Jerusalem). Autoantigens were found in immune complexes from breast and ovarian cancer patients. A new gp40 antigen was found in immune complexes from Burkitt lymphoma and nasopharyngeal carcinoma patients. Also, a new membrane component recognized by EBV-immune T-cells was detected in EBV-transformed cells.

Y. Gazit (Sheba Medical Center, Tel-Hashomer) has detected a specific gp70 antigen on B-cell leukemia cells using monoclonal antibodies that could be a useful diagnostic marker. The generation of monoclonal antibodies for detection of other tumor-specific antigens is being extensively studied by Z. Eshhar (Department of Chemical Immunology, Weizmann Institute) and C. Moroz (the Rogoff-Wellcome Medical Research Institute, Beilinson Medical Center, Petah-Tiqva), who are studying the identification of ferritin-bearing lymphocytes in the circulation of breast cancer and Hodgkins' disease patients.

Approaches to Therapy. There is more interest in adoptive tumor immunotherapy, using T cytotoxic and/or T helper cells sensitized *in vitro* to the tumor and propagated in culture with IL-2. D.W. Weiss and T. Kedar (Department of Immunology, Hebrew University, Hadassah Medical School, Jerusalem) have observed a synergistic therapeutic effect in mice with advanced lung and mammary carcinoma, following conjoint treatment with subcurative chemotherapy. IL-2 propagated lymphocytes and IL-2. A similar effect was observed in nude mice implanted with human tumors. Preliminary clinical trials have been conducted in patients with melanoma and breast cancer by injecting autochthonous lymphoid cells that had been grown in IL-2 into discrete tumor foci. A large proportion of these patients have shown regression of the treated foci following treatment.

Some insight into the mechanisms of activity of a particular immunotherapeutic mycobacterial MER fraction was pro-

vided by findings showing potentiation of macrophage and T-cell function in the development of antibody response *in vitro* and prevention of suppression by T-cell factor *in vivo* by D.W. Weiss and S. Ben-Efraim (Department of Human Microbiology, Tel-Aviv University).

The interaction between the host's antitumor immune response and chemotherapy is also being investigated by S. Ben-Efraim who has found that chemotherapeutic drugs can be classified as immunomodulating and nonimmunomodulating. This classification is based on their ability to promote development of the host's antitumor response and their selective effect on human and murine suppressor T-cells.

Attempts to increase immunogenicity of tumor cells have been made by modulating the membrane lipid composition of tumor cells (M. Shinitzky, Membrane Research Department, Weizmann Institute), and clinical trials of immunotherapy with lipid enriched tumor cells have begun. Thymus humoral factor (THF) is being studied by N. Trainin (Department of Cell Biology, Weizmann Institute), and progress has been made on the chemical characterization and synthesis of the active components of THF mechanisms of potentiation of T-cell-dependent responses and the effect of exogenous THF in tumor-bearing animals.

Another line of research is site-directed chemotherapy with monoclonal antibodies against tumor-cell components conjugated to chemotherapeutic drugs (R. Arnon, E. Hurwitz, and M. Sela (Department of Chemical Immunology, Weizmann Institute). Adriamycin and other cytotoxic drugs have been shown to have a specific effect on neoplastic cells when employed in this way.

Relative to its size, Israel has many groups studying the cellular aspects of the immune response. Studies cover a wide range of subjects, from the development and maturation of immunocytes, through functional and molecular analysis of lymphoid cell subpopulations and their products, to understanding normal and pathological processes. The subject of autoimmunity is being

actively pursued by Israeli scientists, with the Weizmann Institute of Science contributing a large share of Israel's efforts in autoimmunity. Another area under intensive study at both basic and applied levels is that of transplantation and tissue-typing. Three major academic centers in Israel are involved in this research, and all have programs in kidney transplantation. These centers are the Beilinson Medical Center in Petah-Tiqva (Tel-Aviv), the Chaim Sheba Medical Center in Tel Hashomer, and the Hadassah University Hospital in Jerusalem.

Clinical immunology has now been recognized as a distinct discipline by the Israel Medical Association and Ministry of Health. Recognized centers for training programs in this discipline have also been set up around the country. The services provided by such centers are interdisciplinary, with special emphasis on allergic, rheumatic and immunodeficiency diseases. They usually provide all routine immunologic workup. Table 1 lists the variety of clinically oriented immunological research pursued by Israeli scientists and the institutions involved.

#### Applied Immunology

Pioneering studies on synthetic antigens and vaccine were carried out in the laboratory of M. Sela and R. Arnon at the Weizmann Institute. The availability of synthetic antigens has permitted a systematic elucidation of the molecular basis of antigenicity, including such variables as chemical composition, size, shape, availability of certain areas within the antigenic molecules, electrical charge, and optical configuration of the component building blocks. Also, it allowed a similar approach to the study of other immunological phenomena such as tolerance, delayed hypersensitivity, antigenic competition, etc. The use of synthetic antigens, which are antigenically simple, in studies in inbred mice, which are genetically simple, was particularly effective in establishing the genetic control of immune response and its link to the major his-

to compatibility region of the species, as well as the specificity of B- and T-cells. Furthermore, it allowed a conceptual approach to production of vaccines.

The development was based on the initial finding that it is possible to synthesize a peptide, corresponding to a protein fragment, that will lead to antibodies capable of recognizing a conformation-dependent region of the native protein--e.g., lysozyme. Further studies have shown that the same approach can be applied to viruses, using synthetic fragments of the relative viral proteins. The fact that totally synthetic materials could serve for the induction of antiviral immunity is being used as the basis for the production of synthetic vaccines with built-in adjuvanticity. Extensive investigations have been carried out throughout the world (including the Weizmann Institute) in an effort to chemically synthesize vaccines against infectious agents such as diphtheria, cholera, hepatitis B, foot and mouth disease, influenza, polio, and even parasites such as malaria. These vaccines that elicit neutralizing antibodies and *in vivo* protection against challenge may lead to multivalent cross-strain protective vaccines in the future, according to M. Sela.

Biotechnological advances have also been made in Israel in the production of commercial vaccines. The Israel Institute for Biological Research, Rehovot, has developed several veterinary vaccines and produces them in their biofermenters for the local pharmaceutical industry as well as for export. In addition, it is an established reference laboratory for immunodiagnostics of problematic infections, such as rickettsioses, Legionnaires disease, leptospirosis, and human mycoses.

Today, 10 years after somatic cell hybridization was established as a useful procedure for the generation of antibodies of predefined specificity, it has become the approach of choice for the preparation of monoclonal antibodies (mAbs) and is useful in almost every facet of biological research. The relative

Table 1

## Israeli Groups Active in Clinically Oriented Immunological Research

<u>Subject</u>	<u>Investigator</u>	<u>Institution</u>
DNA alterations and autoimmunity in SLE	D. Golan	Technion Medical School
B-cell maturation and immunodeficiency	S. Pollack	Technion Medical School
Human-human hybridomas	R. Baer	Technion Medical School
Drug-induced SLE	A. Kajman	Meir Hospital
	J. Holoshitz	Kfar Soba
Lymphoid cell lines	L.A. Rozensjan	Kfar Soba
Lymphoproliferative disorders: autoimmunity and allergy	A. Pick	Beilinson Medical Center
Human monoclonal antibodies	Y. Shoenfeld	Beilinson Medical Center
Markers of breast cancer	C. Moroz	Beilinson Medical Center
Immunodeficiency	Y. Danon	Beilinson Medical Center
Immunotherapy of breast cancer and melanoma	A. Adler	Beilinson Medical Center
Immunomodulation	B. Shohat	Beilinson Medical Center
Lymphoid populations and use of THF	R. Zaizov	Beilinson Medical Center
	T. Umiel	Beilinson Medical Center
Human MHC	E. Gazit	Sheba Medical Center
Parasitic infection	J. Passwell	Sheba Medical Center
Lymphoproliferative disorders	B. Ramot	Sheba Medical Center
Autoimmune diseases and cryoglobulins	Y. Yust	Ichilov Medical Center
	A. Tanai	Ichilov Medical Center
	Y. Levo	Ichilov Medical Center
Lymphocyte maturation factors	B. Sredni	Bar-Ilan University
Tumor markers and leukotrienes	A. Fink	Kaplan Hospital
Genetic aspects of allergic diseases	C. Geller-Bernstein	Kaplan Hospital
AIDS	Z. Handzel	Kaplan Hospital
Antigen-specific T-cell factors	Z. Weisman	Kaplan Hospital
Interferons	S. Levin	Kaplan Hospital
Coeliac disease	A. Ashkenazi	Kaplan Hospital
Allergic disease	A. Ben-Zvi	Hadassah Medical Center
	B. Sela	Hadassah Medical Center
Neuroimmunology	M. Quastel	Seroka Medical Center
Viral immunity	B. Razar	Seroka Medical Center
Tumor immunity	S. Sagal	Seroka Medical Center
	O. Kuperman	Seroka Medical Center

ease of their production in large quantities and the advantages manifested by their strict specificity and reproducibility have made mAbs also a very lucrative product for clinical and industrial use. In Israel, almost every university and research institute fosters research groups that specialize in the production of mAbs, many of which are already manufactured by the developing biotechnology

industry. Also, these companies have established in-house units that further develop and process the antibodies prepared in the research institutes and develop many new antibodies.

At the Weizmann Institute, the laboratory of Z. Eshhar was instrumental in the development of several mAbs, many of which were prepared in collaboration with other institute members. In the

past 7 years, mAbs have been prepared against small haptens, synthetic antigens, steroid and peptide hormones, cell surface antigens of normal and malignant cells, receptors to hormones and growth factors, cytoskeletal elements, viruses, bacteria, and parasites. During these studies, efficient protocols have been established for immunization with complex mixtures that contain minute amounts of the desired immunogen. Among the mAbs that have been developed at the Weizmann Institute and are being manufactured by the high technology industry (either in bulk or as immunodiagnostic kits) are antibodies against IgE, steroid hormones and their  $\alpha$ -glucuronide derivatives, cytoskeletal elements such as the various elements of the intermediate filaments, tumor-associated antigens such as carcinoembryonic antigen (CEA), and human  $\alpha$ -,  $\beta$ -,  $\gamma$ -interferons. At the Israel Institute for Biological Research, Rehovot, mAbs for several antigens such as CEA, virus, bacteria, and surface markers of normal and activated lymphocytes were developed and are used as immunodiagnostic tools.

At the Hebrew University, Hadassah Medical School, in Jerusalem, M. Schlesinger and associates have developed mAbs against surface markers of human T-cell subpopulations. D. Eliat, in a limited analysis of the antigenic determinants that are immunogenic in autoimmune disease, has generated mAbs specific to various epitopes of nucleic acids and nuclear proteins and studies their role in autoimmune diseases. At Tel-Aviv University, I. Witz and coworkers are generating natural antitumor mAbs. E. Kedar has established hybridomas that produce antibodies that recognize antigenic determinants on protein shared between mouse and human retrovirus that has been presumed to be associated with breast cancer. Y. Haimovich is studying the *in vivo* antitumor effect of mAbs prepared against idiotopic determinants of B-cell lymphomas.

Significant research in antihuman mAbs is being made by research and clinical laboratories affiliated with various hospitals in Israel. For example, C.

Moroz (Peilinson Hospital) has developed mAbs against oncofetal ferritin that serve as a diagnostic tool in breast cancer and Hodgkins' disease. A. Bartal (Rambam Hospital, Haifa) has made various mAbs to breast cancers that help define and stage the malignant tissue.

The budding biotechnology industry in Israel today is also actively involved in research and development of new mAbs. Some already serve as end products, and some are incorporated into diagnostic kits. Thus, Bio-Yeda Ltd., Rehovot, markets a broad range of monoclonal antibodies, including anti-immunoglobulin and antipeptide hormones. Inter-Yeda Ltd., Rehovot, develops mAbs and kits against different steroid hormones, growth hormones and factors, anti-interferon mAb, and antiprekeratin mAb. International Diagnostic Laboratories Ltd. in Jerusalem develops antibodies to thyroid hormones and to microorganisms associated with infectious diseases.

The most exciting research in the field of mAbs is the development of technology for the generation of human mAbs. The group of M. Steinitz at Hadassah Hospital, Jerusalem, is a leader in the derivation of antigen-specific human mAbs from B-cells transformed by Epstein-Barr virus. Among the antibodies produced are anti-D (Rh blood group), antigroup A streptococcal carbohydrate, and antirheumatoid factor. Y. Shoenfeld (Beilinson Medical Center, Tel Aviv) has fused human lymphoid cell lines with lymphocytes from lupus patients to generate anti-DNA and antihistone mAbs. He also applies the same technology for production of human-human hybridomas from patients with breast cancer. In the Biological Research Institute, N. Epstein has produced human-human hybrid lines secreting either lymphokines (IL-2, BCGF) or specific antibodies. In attempts to derive a better human fusion partner, H. Avraham and Z. Eshhar at the Weizmann Institute have established a new human myeloma cell line that serves for the generation of human-human hybridomas producing human monoclonal antibodies.

S. Margel (Weizmann Institute) has developed a new approach for the use of both polyclonal and monoclonal antibodies. The method employs agarose acrobeads (polyacrolein microspheres encapsulated in agarose matrix) that range in size from 0.2  $\mu$ m to 0.8 mm. Due to their high aldehyde contents, such microspheres can covalently bind, in a single step, various ligands with primary amino groups, including antibodies, thus becoming very useful reagents. Bound with the appropriate antibodies, the microspheres with 0.2- $\mu$ m diameter serve as an effective tool for labeling cell surface receptors on either human or mouse lymphocytes. The agarose beads of 150- to 250- $\mu$ m diameter serve for rapid and effective cell fractionation, in which the viability of the cells is unaffected. Larger agarose acrobeads of 0.5 to 0.8 mm have provided the basis for *in vitro* hemoperfusion for specific direct removal of antigens or toxic materials from blood.

Immunotargeting of drugs is another applied research area being pursued actively in Israel. Monoclonal antibodies and specific polyclonal antibodies can be used as carriers of cytotoxic drugs or toxins for immunotargeted chemotherapy. The basic assumption is that antibodies may specifically and exclusively react with particular antigens including those present on tumor cells. This allows specific targeting of chemotherapeutic drugs which also eventually affect normal cells when administered systematically. In appropriately designed conjugates of drugs with specific antibodies, such antibodies can deliver either radioisotopes (immunoimaging for diagnostics) or toxic substances (for immunotherapy) to the tumor cells in a site-specific manner. R. Arnon and M. Sela (Weizmann Institute) are using cytotoxic drugs, such as daunomycin, adriamycin, methotrexate, and platinum compounds conjugated to appropriate antibodies. This procedure has augmented the activity of these drugs and reduced their general toxicity, thus indicating the high potential of this method for therapeutic approaches.

The role of cytokines, such as lymphokines, monokines, and interferons, in the regulation of the immune response is one of the most actively explored aspects of immunology in the world today. It is particularly active in Israel. This research involves intense collaboration between the biotechnological industry and basic science. L. Sachs and colleagues at the Weizmann Institute have been studying soluble macromolecular factors that stimulate cell growth and differentiation. Recently, this group has concentrated on the analysis of the response of leukemic cells to these factors. They have observed two important aspects of malignant transformation which can be illuminated by the study of cytokine functions: the uncoupling of differentiation from growth control in the transformed cell, and ways by which a normal pattern of differentiation can be imposed on transformed cells, using differentiation-inducing cytokines. Large-scale production of two of these stimulating factors, IL-3 and GM-CSF, by recombinant DNA technology is being developed by H. Aviv and colleagues at the Weizmann Institute in collaboration with Biotechnology General Ltd. in Rehovot.

Growth and differentiation factors that support the *in vitro* culturing and maturation of lymphocytes are being studied at Bar Ilan University, Ramat-Gan (groups of L. Rosenszajn and R. Sredni), and the Hebrew University, Hadassah School of Medicine, Jerusalem (R. Kaempfer and A. Treves), both in mouse and in human systems. Human IL-2 and BCGF are being produced today on a large scale at the Israel Institute for Biological Research, Rehovot, by H. Rosenberg, Y. Gozes, N. Epstein, and D. Kobilier. IL-2 is obtained from cultures of lectin-stimulated peripheral blood leukocytes and BCGF from primary leukocytes cultures and cultures of human T-cell hepridomas that produce it constitutively.

Inter-Yeda Ltd. is collaborating with the research group of D. Wallach (Weizmann Institute) in applying recombinant DNA technology for large-scale

production of cytotoxins and exploring the use of these compounds in therapy. This is particularly useful in conjunction with the interferons produced by Inter-Yeda and the use of mAbs to cytotoxins for establishing immunoassays for *in vivo* formation of cytotoxins in disease.

The function of interferons (IFNs) has been studied extensively by several research groups in Israel, particularly at the Weizmann Institute by M. Revel's group. Most of the studies done also in Bar Ilan, University, Ramat-Gan, the Hebrew University, Hadassah Medical Center, Jerusalem, and the Faculty of Agriculture, Hebrew University, Rehovot, have been concentrating on the antiviral effects of interferon. Solid evidence for the IFN's immunoregulatory function, independent of its antiviral activity, was first obtained by D. Wallach et al. They showed that IFN- $\gamma$  induces in cells an increased synthesis of HLA antigens at concentrations that are significantly lower than those necessary for induction of the antiviral state. IFN  $\alpha$ ,  $\beta$ , and  $\gamma$  are being produced by large-scale recombinant DNA technology by Inter-Yeda Ltd., Rehovot, using genes isolated by the research group of M. Revel et al. This company also produces and markets native IFN- $\beta$  induced in human foreskin fibroblast cultures. Native IFN- $\gamma$  induced in cultures of peripheral blood leukocytes and of lymphoblastoid cells is produced at the Israel Institute of Biological Research, Rehovot (H. Rosen-

berg, A. Mizrahi, and A. Traub). Bovine recombinant IFN- $\gamma$  is also produced at the Israel Institute for Biological Research, Rehovot (IIBR) by A. Shafferman and colleagues. Therapeutic applications of the native IFN- $\beta$  produced by Inter-Yeda has been explored extensively, and it is already being applied successfully in several medical centers in Israel for treating viral infections of the eye such as adenovirus epidemic keratoconjunctivitis, herpes-varicella zoster in immuno-depressed patients, as well as for labial and genital herpetic skin lesions and condylomata accuminata. Native IFN- $\alpha$  produced by IIBR has proved useful in treating acute viral hepatitis and laryngeal papillomatosis in the treatment of hairy cell leukemia.

#### Conclusion

Immunology research, both basic and applied, is a major research emphasis in Israel. Israeli scientists are contributing significantly to our knowledge of the many facets of immunological phenomena, including the molecular basis of antigenicity, establishment of the determinant-specific genetic controls of immune response, and immunological unresponsiveness. These scientists have achieved international recognition for their research. Furthermore, collaborative projects between the academic world and the new biotechnological industries have resulted in products for human use and for diagnostic methods.

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